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Plasma Hyaluronidase Activity in Mucolipidoses II and III: Marked Differences From Other Lysosomal Enzymes

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A nearly pathognomonic finding of the lysosomal storage disorders mucolipidoses II and III is the marked increase of plasma lysosomal enzyme activities. The genetic lesion in ML II and III causes defective function of the enzyme UDP-GlcNAc:lysosomal enzyme N-acetylglucosamine-l-phosphotransferase. Defective function of this enzyme results in deficient phosphorylation of lysosomal enzyme asparagine-linked oligosaccharides and a consequent misrouting of many newly synthesized lysosomal enzymes. These enzymes are secreted from cells instead of being targeted to lysosomes, with resultant marked elevations of multiple lysosomal enzyme activities in plasma. We report here that plasma hyaluronidase activity, an endoglycosidase of presumably lysosomal origin, is not increased in the plasma from individuals with mucolipidoses II and III, unlike most lysosomal enzymes. Our data suggest the possibility that hyaluronidase is not targeted to lysosomes by a lysosomal enzyme phosphosmannosyl recognition mechanism. Alternatively, hyaluronidase activity may not be present in the cell type(s) responsible for the lysosomal enzyme hypersecretion in mucolipidoses II and III which, along with its deficiency in fibroblasts and leukocytes, would constitute an unusual tissue distribution of activity for a soluble lysosomal enzyme.

KEY WORDS: lysosomal enzymes, mucolipidosis, hyaluronidase

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INTRODUCTION

The lysosomal storage disorders mucolipidoses II and III (ML II and III), also known as I-cell disease and pseudo-Hurler polydystrophy, are autosomal recessive conditions caused by a deficiency of the enzyme UDP-GlcNAc:lysosomal enzyme N-acetylglucosamine-l-phosphotransferase [Kornfeld and Sly, 1995]. This enzyme is critical for the normal intracellular trafficking of most lysosomal enzymes, initiating the synthesis of specific phosphorylated oligosaccharides on lysosomal enzymes that can then engage phosphomannosyl receptors and subsequently transit to lysosomes [Varki, 1992]. Absent or defective function of this enzyme causes marked intracellular deficiencies of most lysosomal enzyme activities in cultured fibroblasts from individuals with ML II and III [Leroy and DeMars, 1967; Leroy et al., 1972; Kelly et al., 1975]; this is accompanied by increased secretion of these enzymes into the culture medium [Wiesmann et al., 1971a]. One of the in vivo correlates of this is the markedly increased concentration of most plasma and urinary lysosomal enzyme activities. Plasma lysosomal enzyme activities are typically increased from one to over two orders of magnitude in ML II and ML III compared to controls [Wiesman et al., 1971b; Kelly et al., 1975; Herd et al., 1978; Ward et al., 1993].

Hyaluronidase is an endoglycosidase that cleaves 1β4-N-acetylhexosamide linkages in several glycosaminoglycans: hyaluronic acid, chondroitin, chondroitin 4-sulfate, and chondroitin 6-sulfate [Meyer and Rapport, 1952; Meyer, 1971; Barrett and Heath, 1977; Kreil, 1995]. The enzyme has an acidic pH optimum and is present in a lysosome-enriched fraction in many tissues including bone, brain, gingiva, liver, spleen, and submandibular gland [Hutterer, 1966; Aronson and Davidson, 1967; Vaes, 1967; Goggins et al., 1968; Tan and Bowness, 1968; Margolis et al., 1972; Barrett and Heath, 1977]. Because of its acidic pH optima and subcellular localization, hyaluronidase is considered a lysosomal enzyme in most tissues, except for testicular and sperm hyaluronidase [Barrett and Heath, 1977; Lin et al., 1994]. The enzyme present in serum has sim-

ilar properties to the lysosomal hyaluronidases from other tissues and differs from testicular and sperm hyaluronidase activities and is therefore presumed to be of lysosomal origin [De Salegui et al., 1967; Stephens et al., 1976; Barrett and Heath, 1977; Afify et al., 1993; Kreil, 1995].

Several lines of evidence establish that there are multiple mechanisms for targeting different lysosomal enzymes, some of which are enzyme specific and some of which may be tissue specific [Kornfeld and Mellman, 1989; Varki, 1992]. There is no published information regarding the biosynthesis and intracellular transport of human lysosomal hyaluronidase. As a first question in studying the biosynthesis and regulation of this enzyme, we decided to determine whether it is targeted to lysosomes like most other soluble lysosomal enzyme activities, i.e., via a phosphomannosyl recognition pathway. A genetic approach to experimentally address this issue is to assess whether hyaluronidase is affected by the genetic lesion in mucolipidoses II and III in a similar fashion to most other lysosomal enzyme activities and has a markedly increased concentration in plasma specimens from individuals with ML II and III. Our results indicate that this is not the case and raise the possibility that human hyaluronidase is targeted to lysosomes by a non-phosphomannosyl dependent mechanism.

METHODS

Specimens from individuals with ML II and ML III were obtained from the Division of Medical Genetics, The Shriver Center, Waltham, MA: samples from control individuals were obtained by informed consent from normal healthy persons participating in the Tay-Sachs Disease Prevention Program of that center. Serum and plasma hyaluronidase activities were assayed using human umbilical cord hyaluronic acid as substrate (Sigma Chemical Co., St. Louis, MO). Ten microliters of serum or plasma were incubated for 4 hours at 37°C with 0.25 mL buffered substrate solution (0.1 M sodium formate, pH 3.9, containing 0.1 M NaCl, 250 μg/mL hyaluronic acid, and 1.5 mM saccharic acid 1,4-lactone) [Natowicz and Wang, 1996]. The reaction was terminated with borate buffer and reducing N-acetylglucosamine termini were determined by the Reissig reaction [Reissig et al., 1955; Natowicz and Wang, 1996]. Blanks for the hyaluronidase reaction consisted of tubes in which the buffered substrate was incubated in the absence of serum or plasma and which subsequently received borate buffer, then serum, and were then immediately boiled. Mixing experiments were done by combining varying amounts of patient and control plasma specimens, keeping the total plasma volume constant at ten microliters. Plasma alpha-mannosidase activity was determined using the 4-methylumbelliferyl alpha-D-mannopyranoside substrate as described previously [Prence and Natowicz, 1992]. Total plasma beta-hexosaminidase activity (beta-hexosaminidases A and B) and quantitation of the two isozymes was determined using 4-methylumbelliferyl N-acetyl-beta-D-glucosaminide as substrate; the percent of the B isozyme was determined as the percent of activity that was thermostable after heating the specimen at 52°C [Natowicz et al., 1991]. Plasma beta-glucuronidase activity was determined by incubating 40 uL sample in a reaction volume of 100 uL with 5 mM 4-methylumbelliferyl beta-D-glucuronide and 0.1 M citrate-phosphate buffer pH 4.5 for 30 min at 37°C. The reaction was terminated and fluorescence measured as described [Prence and Natowicz, 1992]. For all enzymes, a unit of activity represents the production of a micromole of reaction product per min at 37°C.

RESULTS

Table I shows the expected 10- to more than 300-fold increases in alpha-mannosidase, beta-glucuronidase, and beta-hexosaminidase activities in the plasma of five individuals with ML II and ML III. Unexpectedly, the mean plasma hyaluronidase activity in these same individuals was 2,872 mU/L (range: 1,453–3,874 mU/L; S.D., 852 mU/L), not elevated compared to the plasma hyaluronidase activity of 100 normal controls (mean: 4,476 mU/L; range: 2,488–9,051 mU/L; S.D.: 1,144 mU/L) (Table I). A mixing experiment using varying amounts of control and ML III plasmas showed no evidence of a soluble inhibitor of hyaluronidase activity in ML III plasma (Table II). Hyaluronidase activity was not detected in control human leukocytes, fibroblasts, or fibroblast secretions (data not shown).

TABLE I. Plasma Lysosomal Enzyme Activities in ML II and ML III

Individual/ diagnosis	β-Hex A and B[a]	% Hex B[b]	β-Gluc[c]	α-Man[d]	HA-ASE[e]
#1/ML II	295,273	57.4	77,655	35,070	2,572
#2/ML III	538,809	61.9	40,247	77,321	3,874
#3/ML III	638,191	62.6	30,895	55,611	3,624
#4/ML II	580,743	57.0	69,305	13,360	1,453
#5/ML III	205,577	59.0	41,416	37,241	2,839
Normal controls (mean ± SD)	19,823 ± 6,229	32.7 ± 4.2	701 ± 317	200 ± 67	4,476 ± 1,144

[a] Total plasma beta-hexosaminidase A and B activities; all enzyme activities expressed as mU/L.
[b] The % Hex B is the thermostable fraction of the total beta-hexosaminidase activity as described in Methods.
[c] Beta-glucuronidase activity.
[d] Alpha-mannosidase activity.
[e] Hyaluronidase activity.

TABLE II. Mixing Experiment to Assess Possible Hyaluronidase Inhibitors

Ratio of ML III:Control	Expected activity[a]	Observed activity
Patient 2:Control		
100:0		3,758
75:25	5,060	5,194
50:50	6,379	6,396
25:75	7,699	7,966
0:100		9,001
Patient 3:Control		
100:0		3,123
75:25	4,593	5,043
50:50	6,062	6,146
25:75	7,548	8,049
0:100		9,001

[a] Aliquots of ML III and control plasma specimens were added at the indicated ratios and the hyaluronidase activity assayed as described in Methods. The expected hyaluronidase activity refers to the amount of activity expected if there is no inhibitor(s) present in ML III plasma. Hyaluronidase activity is expressed as mU/L.

DISCUSSION

A defining and almost pathognomonic feature of mucolipidoses II and III is the marked increase of multiple plasma lysosomal enzyme activities in individuals with those conditions [Wiesmann et al., 1971a,b; Kelly et al., 1975; Herd et al., 1978]. The increased extracellular concentrations of these enzymes is a consequence of the enzymatic deficiency in those conditions that, in turn, results in the misrouting of lysosomal enzymes, although the tissue(s) of origin of the plasma lysosomal enzymes in individuals with ML II and III has not been established.

Unlike most of the other lysosomal enzymes, there was no increase in the concentration of hyaluronidase activity in the plasma of any of the individuals with ML II or III. There are several possible explanations for this. First, it is possible that this enzyme is not a lysosomal enzyme. This possibility is unlikely in view of the shared biochemical properties of this enzyme with known lysosomal hyaluronidases, especially its markedly acidic pH optimum which is a hallmark of most lysosomal enzymes [Natowicz and Wang, 1996]. Furthermore, we recently identified an individual with a histologically and ultrastructurally confirmed lysosomal storage disorder who lacks plasma hyaluronidase activity and whose parents have approximately half normal concentrations of the enzyme. These findings strongly implicate a lysosomal localization of this enzyme and an autosomal recessive mode of inheritance for hyaluronidase deficiency (Natowicz et al., manuscript submitted).

Second, it is possible that there is in fact an elevation of hyaluronidase activity in the plasma from individuals with ML II and III but that this increased activity was not detected by the assays used. This possibility is unlikely in view of the absence of inhibition of hyaluronidase activity in control plasma when mixed with ML III plasma specimens.

Third, it is possible that hyaluronidase utilizes a different intracellular routing mechanism from the well-described phosphomannosyl-dependent pathways [Kornfeld and Mellman, 1989; Varki, 1992; Kornfeld, 1992]. Alternative pathways for the targeting of lysosomal enzymes clearly exist. Some cells and tissues in ML II, such as circulating granulocytes, brain, kidney, liver, and spleen, have normal levels of lysosomal enzymes [Wiesmann et al., 1971b; Leroy et al., 1972; Kato et al., 1979; Owada and Neufeld, 1982; Waheed et al., 1982]. ML II and III lymphoblasts also have substantial intralysosomal concentrations of several lysosomal enzymes [Miller et al., 1993; Glickman and Kornfeld, 1993]. In addition, some lysosomal proteins are not deficient in ML II or III fibroblasts, implying that those proteins do not require the phosphomannosyl recognition marker pathway for trafficking to lysosomes in any cell type [Leroy and DeMars, 1967; Leroy et al., 1972; Herd et al., 1978; Varki, 1992; Ward et al., 1993]. Other lines of evidence also indicate the existence of alternative pathways for directing proteins to lysosomes [Gonzalez-Noriega et al., 1989; Kornfeld and Mellman, 1989; Klionsky and Emr, 1990; Rijnboutt et al., 1991]. If the targeting of hyaluronidase to lysosomes does not involve a phosphomannosyl recognition marker, then the enzyme would be appropriately targeted to lysosomes in the cells of individuals with ML II or ML III and there would not be increased secretion of hyaluronidase in the blood.

We did not detect any hyaluronidase activity in leukocytes, fibroblasts, or fibroblast secretions. Others have also noted an absence of hyaluronidase activity in cultured human skin fibroblasts [Arbogast et al., 1975; Klein and von Figura, 1980]. This deficiency of hyaluronidase activity in both leukocytes and fibroblasts is an unusual if not unprecedented pattern of expression of a human lysosomal enzyme. Consequently, an alternative explanation for our results is that hyaluronidase does utilize a phosphomannosyl-dependent targeting pathway but that the lack of the expected increase of hyaluronidase activity in the plasma of individuals with ML II and III is the result of a deficiency of hyaluronidase activity in the cell type(s) that is (are) primarily responsible for the marked increases of other plasma lysosomal enzymes in ML II and III. If, for example, either fibroblasts or circulating lymphocytes is a major source of the plasma lysosomal enzyme activities in ML II and III, one would expect that the plasma hyaluronidase activity in ML II and III to be normal since neither of these cell types has detectable hyaluronidase activity. The liver is not a candidate tissue in this regard since there is substantial hyaluronidase activity in human liver [Gold, 1982; Natowicz, unpublished]. Additional studies are underway to discriminate between these interesting possibilities.

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REFERENCES

Afify AM, Stern M, Guntenhoner M, Stern R (1993): Purification and characterization of human serum hyaluronidase. Arch Biochem Biophys 305:434–441.

Arbogast B, Hopwood JJ, Dorfman A (1975): Absence of hyaluronidase in cultured human skin fibroblasts. Biochem Biophys Res Commun 67:376–382.

Aronson NN, Davidson EA (1967): Lysosomal hyaluronidase from rat liver. J Biol Chem 242:437–440.

Barrett AJ, Heath MF (1977): Lysosomal enzymes. In Dingle JT (ed): "Lysosomes: A Laboratory Handbook." New York: Elsevier, pp 19–146.

De Salegui M, Plonska H, Pigman W (1967): A comparison of serum and testicular hyaluronidase. Arch Biochem Biophys 121:548–554.

Glickman JN, Kornfeld S (1993): Mannose 6-phosphate-independent targeting of lysosomal enzymes in I-Cell disease B lymphoblasts. J Cell Biol 123:99–108.

Goggins JF, Fullmer HM, Steffek AJ (1968): Hyaluronidase activity of human gingiva. Arch Pathol 85:272–274.

Gold EW (1982): Purification and properties of hyaluronidase from human liver. Biochem J 205:69–74.

Gonzalez-Noriega A, Coutino R, Saavedra VM, Barrera R (1989): Adsorptive endocytosis of lysosomal enzymes by human fibroblasts: Presence of two different functional systems that deliver an acid hydrolase to lysosomes. Arch Biochem Biophys 268: 649–658.

Herd JK, Dvorak AD, Wiltse HE, Eisen JD, Kress BC, Miller AL (1978): Mucolipidosis type III: Multiple elevated serum and urine enzyme activities. Am J Dis Child 132:1181–1186.

Hutterer F (1966): Degradation of mucopolysaccharides by hepatic lysosomes. Biochim Biophys Acta 115:312–319.

Kato E, Yokoi T, Taniguchi N (1979): Lysosomal acid hydrolases in lymphocytes of I-cell disease. Clin Chim Acta 95:285–290.

Kelly TE, Thomas GH, Taylor HA, McKusick VA, Sly WS, Glaser JH, Robinow M, Luzzatti L, Espiritu C, Feingold M, Bull MJ, Ashenhurst EM, Ives EJ (1975): Mucolipidosis III (pseudo-Hurler polydystrophy): Clinical and laboratory studies in a series of 12 patients. Johns Hopkins Med J 137:156–175.

Klein U, von Figura K (1980): Characterization of dermatan sulfate in mucopolysaccharidosis VI: Evidence for the absence of hyaluronidase-like enzymes in human skin fibroblasts. Biochim Biophys Acta 630:10–14.

Klionsky DJ, Emr SD (1990): A new class of lysosomal/vacuolar protein sorting signals. J Biol Chem 265:5349–5352.

Kornfeld S (1992): Structure and function of the mannose 6-phosphate/insulin growth factor II receptors. Annu Rev Biochem 61:307–330.

Kornfeld S, Mellman I (1989): The biogenesis of lysosomes. Annu Rev Cell Biol 5:483–525.

Kornfeld S, Sly WS (1995): I-cell disease and pseudo-Hurler polydystrophy: disorders of lysosomal enzyme phosphorylation and localization. In Scriver CR, Beaudet AL, Sly WS, Valle D (eds): "The Metabolic and Molecular Basis of Inherited Disease." New York: McGraw-Hill, pp 2495–2508.

Kreil G (1995): Hyaluronidases: A group of neglected enzymes. Protein Science 4:1666–1669.

Leroy JG, DeMars RI (1967): Mutant enzymatic and cytological phenotypes in cultured human fibroblasts. Science 157:804–806.

Leroy JG, Ho MW, MacBrinn MC, Zielke K, Jacob J, O'Brien JS (1972): I-cell disease: Biochemical studies. Pediatr Res 6:752–757.

Lin Y, Mahan K, Lathrop WF, Myles DG, Primakoff P (1994): A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg. J Cell Biol 125:1157–1163.

Margolis RU, Margolis RK, Santella R, Atherton DM (1972): The hyaluronidase of brain. J Neurochem 19:2325–2332.

Meyer K (1971): Hyaluronidases. In Boyer PD (ed): "The Enzymes." New York: Academic Press, pp 307–320.

Meyer K, Rapport MM (1952): Hyaluronidases. Adv Enzymol 13: 199–236.

Miller AL, Norton V, Robertson R, Jenks M, Yeh RY, Wright D (1993): Light and heavy lysosomes: characterization of N-acetyl-β-D-hexosaminidase isolated from normal and I-cell disease lymphoblasts. Glycobiol 3:313–318.

Natowicz MR, Prence EM, Cajolet A (1991): Marked variation in blood beta-hexosaminidase in Gaucher disease. Clin Chim Acta 203: 17–22.

Natowicz MR, Wang Y (1996): Human serum hyaluronidase: characterization of a clinical assay. Clin Chim Acta 245:1–6.

Owada M, Neufeld EF (1982): Is there a mechanism for introducing acid hydrolases into liver lysosomes that is independent of mannose 6-phosphate recognition? Evidence from I-cell disease. Biochem Biophys Res Commun 105:814–820.

Prence EM, Natowicz MR (1992): Diagnosis of alpha-mannosidosis by measuring alpha-mannosidase in plasma. Clin Chem 38:501–503.

Reissig JL, Strominger JL, LeLoir LF (1955): A modified colorimetric method for the estimation of N-acetylamino sugars. J Biol Chem 217:959–966.

Rijnboutt S, Kal AJ, Geuze HJ, Aerts H, Strous GJ (1991): Mannose 6-phosphate-independent targeting of cathepsin D to lysosomes in HepG2 cells. J Biol Chem 266:23586–23592.

Stephens RW, Sutherland J, Ghosh P, Taylor TKF (1976): Human serum and synovial fluid hyaluronidase-bovine testicular hyaluronidase is not a valid substitute in drug evaluation studies. Biochem Pharmacol 25:1507–1511.

Tan YH, Browness JM (1968): Canine submandibular-gland hyaluronidase. Biochem J 110:9–17.

Vaes G (1967): Hyaluronidase activity in lysosomes of bone tissue. Biochem J 108:802–804.

Varki A (1992): Role of oligosaccharides in the intracellular and intercellular trafficking of mammalian glycoproteins. In Fukuda M (ed): "Cell Surface Carbohydrates and Cell Development." Boca Raton, FL: CRC Press, pp 25–69.

Waheed A, Pohlmann R, Hasilik A, von Figura K, van Elsen A, Leroy JG (1982): Deficiency of UDP-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-l-phosphotransferase in organs of I-cell patients. Biochem Biophys Res Commun 105:1052–1058.

Ward C, Singh R, Slade C, Fensom AH, Fahmy A, Semrin A, Sjovall A, Talat A, Hasilik A, Klein I, Benson PF (1993): A mild form of mucolipidosis type III in four Baluch siblings. Clin Genet 44:313–319.

Wiesmann UN, Lightbody J, Vassella F, Herschkowitz NN (1971a): Multiple lysosomal enzyme deficiency due to enzyme leakage? N Engl J Med 284:109–110.

Wiesman U, Vassella F, Herschkowitz N (1971b): "I-cell" disease: Leakage of lysosomal enzymes into extracellular fluids. N Engl J Med 285:1090–1091.